Granulocyte Colony-Stimulating Factor Enhances Endotoxin-Induced Decrease in Biliary Excretion of the Antibiotic Cefoperazone in Rats

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We have recently reported that endotoxin (lipopolysaccharide [LPS]) derived from Klebsiella pneumoniae dramatically decreased the biliary excretion of the β-lactam antibiotic cefoperazone (CPZ), which is primarily excreted into the bile via the anion transport system, in rats. The present study was designed to investigate the effect of human recombinant granulocyte colony-stimulating factor (G-CSF), which is reported to be beneficial in experimental models of inflammation, on the pharmacokinetics and biliary excretion of CPZ in rats. CPZ (20 mg/kg of body weight) was administered intravenously 2 h after the intravenous injection of LPS (250 μg/kg). G-CSF was injected subcutaneously at 12 μg/kg for 3 days and was administered intravenously at a final dose of 50 μg/kg 1 h before LPS injection. Peripheral blood cell numbers were also measured. LPS dramatically decreased the systemic and biliary clearances of CPZ and the bile flow rate. Pretreatment with G-CSF enhanced these decreases induced by LPS. The total leukocyte numbers were increased in rats pretreated with G-CSF compared to the numbers in the controls, while the total leukocyte numbers were decreased (about 3,000 cells/μl) by treatment with LPS. Pretreatment with G-CSF produces a deleterious effect against the LPS-induced decrease in biliary secretion of CPZ, and leukocytes play an important role in that mechanism.

Endotoxin (lipopolysaccharide [LPS]), a component of the bacterial cell wall, is known to have various biological and immunological activities and induces acute kidney and liver dysfunctions (14). A number of articles concern the effects of LPS on the pharmacokinetics of drugs have been published (2, 7, 19, 23–25, 30). LPS decreases the renal excretion of drugs (2, 23–25) and impairs P-450-mediated hepatic metabolizing enzyme activities (29, 30). It has also been reported that LPS causes cholestatic jaundice in patients with gram-negative bacterial infections concomitant with elevations in plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (14) and that LPS decreases the biliary excretion of some drugs that are primarily excreted in the bile. In particular, the present study aimed at investigating the effects of pretreatment with G-CSF on the LPS-induced changes in the pharmacokinetics and biliary excretion of CPZ in rats. In an attempt to clarify their mechanisms of action, the role of TNF-α or PMN in these changes is also discussed.

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MATERIALS AND METHODS

Chemicals. CPZ was donated by Toyama Chemical Industries (Tokyo, Japan). Human recombinant G-CSF produced by Chinese hamster ovary cells was kindly supplied by Chugai Pharmaceutical (Tokyo, Japan) in the form of a commercial preparation for injection (Neutrogen). LPS was isolated as described (11, 12) from a cultured mutant strain derived from K. pneumoniae Kasuya (O3:K1) (26). The internal standard 3-butylxanthine was synthesized in our laboratory and was identical to that reported previously (10). All other reagents were commercially available and were of analytical grade.

Animals and experimental protocols. Eight- to 9-week-old male Wistar rats (Nippon SLC, Hamamatsu, Japan) were used in this study. Rats were divided into four treatment groups: (i) the control group (n = 6), (ii) the G-CSF-pretreated group (n = 5), (iii) the LPS-treated group (n = 5), and (iv) the G-CSF- and LPS-treated group (G-CSF-pretreated and LPS-treated group) (n = 5). In the two groups with G-CSF pretreatment, the rats received subcutaneous injections of G-CSF at a dosage of 12 μg/kg of body weight once a day for 3 days before the day of the experiment, and on the day of the experiment they received an intravenous injection of G-CSF at a dose of 50 μg/kg 1 h before LPS administration. LPS dissolved in isotonic saline was administered intravenously into the jugular vein at a dose of 250 μg/kg 2 h before CPZ administration, and saline was injected instead of LPS for the groups not treated with LPS (control and G-CSF-pretreated groups).

To measure the blood cell number, plasma ALT and AST activities, and plasma creatinine concentration, whole blood (0.25 ml) was drawn from the abdominal artery just before CPZ administration while the rats were under ether anesthesia. To examine the kinetics of the leukocyte (WBC) counts after LPS administration, blood samples of about 0.3 ml each were taken from the jugular vein in the control and the G-CSF-pretreated rats. Blood samples were taken 5 min before and 15, 30, 60, 90, and 120 min after LPS administration. The blood cells were counted immediately after sampling, and then plasma samples for the measurement of both liver enzyme and creatinine levels were prepared by centrifugation.

For CPZ clearance experiments, 1 day before the experiments the rats were placed under light pentobarbital anesthesia (25 mg/kg) and were cannulated with polyethylene tubes for bile and urine collection, respectively. All experiments were done while the rats were under ether anesthesia. The effects of LPS on plasma ALT and AST activities and on creatinine concentrations in rats pretreated or not pretreated with G-CSF were determined by dividing the total amounts of CPZ excreted into the bile and into the urine during the collection period (120 min) by the corresponding AUC (AUCtotal = AUCbile + AUCurine).

Plasma, bile, and urine CPZ concentrations were determined by the high-performance liquid chromatography (HPLC) method described previously (10). Briefly, a mixture 50 μl each of sample and 50 μl of phosphate buffer (pH 7.4) containing 3-butylxanthine as an internal standard was deproteinized with 50 ml of phosphate buffer (pH 7.4) containing 3-butylxanthine as an internal standard. The plasma samples were taken 5 min before and 15, 30, 60, 90, and 120 min after LPS administration. The blood cells were counted immediately after sampling, and then plasma samples for the measurement of hepatic enzyme and creatinine levels were prepared by centrifugation.

RESULTS

The peripheral blood cell numbers and hematocrits measured 2 h after LPS or saline administration in four groups are summarized in Table 1. The erythrocyte (RBC) numbers and the percentage of PMNs were significantly increased in the G-CSF-pretreated group compared to those in the control group. On the other hand, the WBC numbers significantly decreased 2 h after the administration of LPS, and the WBC numbers in the LPS-treated and the G-CSF- and LPS-treated groups were similar regardless of their G-CSF pretreatment status. The percentage of PMNs in both the LPS-treated and the G-CSF- and LPS-treated groups, however, was increased. The percentage of PMNs in the G-CSF- and LPS-treated group was significantly different from those in the control group. On the other hand, the WBC numbers immediately decreased to less than 3,000 cells/μl after LPS treatment and seemed to be constant from 60 to 120 min after LPS administration, although the initial WBC number in G-CSF-pretreated rats was significantly larger than those in control rats.

The effects of LPS on plasma ALT and AST activities and on creatinine concentrations in rats pretreated or not pretreated with G-CSF are listed in Table 2. Pretreatment with G-CSF did not change plasma AST activity, but the activity increased significantly in the LPS-treated and the G-CSF- and LPS-treated groups. On the other hand, plasma ALT activity was significantly decreased in the G-CSF-pretreated group compared to that in the control group. In the G-CSF- and LPS-treated group, plasma ALT activity was significantly decreased compared with those in the G-CSF-pretreated and LPS-treated groups.

log concentration-time data. Systemic clearance (CLsys) was determined as CLsys = dose/AUC. Mean residence time (MRT) was calculated as MRT = AUC/MRT. Steady-state volume of distribution (Vss) was calculated as Vss = CLsys × MRT. Biliary clearance (CLbile) and renal clearance (CLR) were determined by dividing the total amounts of CPZ excreted into the bile and into the urine during the collection period (120 min) by the corresponding AUC. All computer analyses were performed with the nonlinear least-squares regression program MULTI, written by Yamaoka et al. (40), by weighing the data with the reciprocal of the concentration.

Statistical analysis. Results were expressed as mean ± standard error. Statistical comparisons among the groups were assessed by Student’s t-test, with the limit of statistical significance (P) being <0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WBC</th>
<th>% Neutrophils</th>
<th>RBC counts</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.38 ± 0.56</td>
<td>11.94 ± 12.9</td>
<td>7.02 ± 0.13</td>
<td>39.52 ± 0.70</td>
</tr>
<tr>
<td>G-CSF</td>
<td>13.81 ± 1.82</td>
<td>24.68 ± 2.13</td>
<td>7.63 ± 0.13</td>
<td>41.10 ± 0.60</td>
</tr>
<tr>
<td>LPS</td>
<td>3.57 ± 0.79</td>
<td>31.02 ± 8.84</td>
<td>7.27 ± 0.15</td>
<td>38.32 ± 0.67</td>
</tr>
<tr>
<td>G-CSF and LPS</td>
<td>2.83 ± 0.48</td>
<td>39.74 ± 5.00</td>
<td>7.09 ± 0.15</td>
<td>38.00 ± 0.67</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± standard error (n = 5).
* Significantly different from the control group (P < 0.05).
* Significantly different from the control group (P < 0.01).

The peripheral blood cell numbers and hematocrits measured 2 h after LPS or saline administration in four groups are summarized in Table 1. The erythrocyte (RBC) numbers and the percentage of PMNs were significantly increased in the G-CSF-pretreated group compared to those in the control group. On the other hand, the WBC numbers significantly decreased 2 h after the administration of LPS, and the WBC numbers in the LPS-treated and the G-CSF- and LPS-treated groups were similar regardless of their G-CSF pretreatment status. The percentage of PMNs in both the LPS-treated and the G-CSF- and LPS-treated groups, however, was increased. The percentage of PMNs in the G-CSF- and LPS-treated group was significantly different from those in the control group. On the other hand, the WBC numbers immediately decreased to less than 3,000 cells/μl after LPS treatment and seemed to be constant from 60 to 120 min after LPS administration, although the initial WBC number in G-CSF-pretreated rats was significantly larger than those in control rats.

The effects of LPS on plasma ALT and AST activities and on creatinine concentrations in rats pretreated or not pretreated with G-CSF are listed in Table 2. Pretreatment with G-CSF did not change plasma AST activity, but the activity increased significantly in the LPS-treated and the G-CSF- and LPS-treated groups. On the other hand, plasma ALT activity was significantly decreased in the G-CSF-pretreated group compared to that in the control group. In the G-CSF- and LPS-treated group, plasma ALT activity was significantly decreased compared with those in the G-CSF-pretreated and LPS-treated groups. The
creatinine concentration in plasma was significantly increased by LPS treatment but it was not changed by G-CSF treatment.

Mean semilogarithmic plasma concentration-time curves for CPZ in the four groups following the intravenous injection of a dose of 20 mg/kg are shown in Fig. 2. No changes in the plasma concentration-time profile of CPZ was observed in the G-CSF-pretreated group. However, remarkable prolongation in the disappearance of CPZ from plasma was observed in LPS-treated and G-CSF- and LPS-treated rats. This prolongation was longer in the G-CSF- and LPS-treated group than that in the LPS-treated group.

The corresponding pharmacokinetic parameters of CPZ in the four groups are summarized in Table 3. No changes in the pharmacokinetic parameters of CPZ was observed in the G-CSF-pretreated group, indicating that G-CSF itself did not influence the disposition of CPZ. On the other hand, LPS slightly increased the $V_{SS}$ of CPZ, although no significant difference was observed between the control and LPS-treated groups. The $CL_{SYS}$ of CPZ was decreased to 56% of that for the control by LPS administration. The decreased magnitude was greater in the G-CSF- and LPS-treated group than in the LPS-treated group. The MRTs of CPZ were also significantly prolonged by LPS administration, and the prolongation was greater in the G-CSF- and LPS-treated group than in the LPS-treated group.

The effects of LPS administration on the cumulative biliary excretion-time courses of CPZ in rats pretreated or not pretreated with G-CSF are shown in Fig. 3. The percentage of the dose excreted in the bile and urine within the experimental period (120 min) in each group is summarized in Table 4. There were no changes in the biliary or urinary excretion of CPZ in the G-CSF-pretreated group. However, the biliary excretion of CPZ was significantly decreased in both the LPS-treated and the G-CSF- and LPS-treated groups. The decrease was greater in the G-CSF- and LPS-treated group than in the LPS-treated group. Correspondingly, significant decreases in the $CL_{BIL}$ of CPZ were observed in the LPS-treated and the G-CSF- and LPS-treated groups compared to those in the control and G-CSF-pretreated groups. Significant differences in the $CL_{BIL}$ of CPZ between the LPS-treated group and the G-CSF- and LPS-treated were also noted. In addition, a significant decrease in the $CL_{UR}$ of CPZ was found in the G-CSF- and LPS-treated group compared to those in the control and G-CSF-pretreated groups, although the percentage of the dose excreted in the urine was significantly increased in the G-CSF- and LPS-treated group.

The bile flow rates following LPS administration in the four groups are shown in Fig. 4. The bile flow rate was relatively constant throughout the experimental period for each treatment group, while significant decreases in the bile flow rate were observed in both the LPS-treated and G-CSF- and LPS-treated groups. The decrease was greater in the G-CSF- and LPS-treated group than in the LPS-treated group, as was the case for other parameters, although no changes in the bile flow rate were observed in the G-CSF-pretreated group.

### Table 2. Effects of LPS on plasma AST and ALT activities and plasma creatinine concentration in rats pretreated or not pretreated with G-CSF

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST concn (IU/liter)</th>
<th>ALT concn (IU/liter)</th>
<th>Creatinine concn (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.13 ± 1.41</td>
<td>11.31 ± 1.09</td>
<td>0.694 ± 0.011</td>
</tr>
<tr>
<td>G-CSF</td>
<td>35.71 ± 1.12</td>
<td>6.95 ± 1.16</td>
<td>0.732 ± 0.064</td>
</tr>
<tr>
<td>LPS</td>
<td>55.76 ± 4.36</td>
<td>9.42 ± 1.79</td>
<td>0.779 ± 0.027</td>
</tr>
<tr>
<td>G-CSF and LPS</td>
<td>52.81 ± 2.84</td>
<td>4.84 ± 0.76</td>
<td>0.745 ± 0.019</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± standard error (n = 5).

### Table 3. Effects of LPS on the pharmacokinetic parameters of CPZ in rats pretreated or not pretreated with G-CSF

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$V_{SS}$ (liter/kg)</th>
<th>$CL_{SYS}$ (liter/h/kg)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.342 ± 0.024</td>
<td>1.621 ± 0.145</td>
<td>0.194 ± 0.014</td>
</tr>
<tr>
<td>G-CSF</td>
<td>0.326 ± 0.022</td>
<td>1.398 ± 0.057</td>
<td>0.213 ± 0.012</td>
</tr>
<tr>
<td>LPS</td>
<td>0.416 ± 0.032</td>
<td>0.908 ± 0.111</td>
<td>0.492 ± 0.093</td>
</tr>
<tr>
<td>G-CSF and LPS</td>
<td>0.306 ± 0.022</td>
<td>0.423 ± 0.076</td>
<td>0.822 ± 0.179</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± standard error (n = 5).

![FIG. 1. Time-dependent changes in WBC counts induced by endotoxin treatment in rats pretreated (●) or not treated (○) with G-CSF. Each plot represents the mean ± standard error (n = 5).](image1)

![FIG. 2. Effect of LPS on concentrations of CPZ in plasma after the administration of a single intravenous dose of 20 mg/kg in rats pretreated or not pretreated with G-CSF. Symbols: ○, control; ●, G-CSF pretreatment; △, LPS treatment; ▲, G-CSF and LPS treatment. Each plot represents the mean ± standard error (n = 3 or 6).](image2)
FIG. 3. Effect of LPS on cumulative excretion of CPZ after the administration of a single intravenous dose of 20 mg/kg within the 120-min collection period in rats pretreated or not pretreated with G-CSF. Symbols: ○, control; ●, G-CSF pretreatment; △, LPS treatment; ▲, G-CSF and LPS treatment. Each plot represents the mean ± standard error (n = 5 or 6).

DISCUSSION

The present study showed that circulating WBC numbers and the percentage of PMNs were increased by pretreatment with G-CSF and that circulating WBC numbers 2 h after administration of LPS were similar for the two groups regardless of G-CSF pretreatment, although the numbers of PMNs did not change (Fig. 1 and Table 1). These results suggested that WBCs are well distributed to LPS-induced inflammatory lesions in rats pretreated with G-CSF. On the other hand, the significant increase in RBC numbers found in the G-CSF-pretreated group is unlikely to be important, since this change was small compared to those in WBC numbers and the percentage of PMNs. It could therefore be considered that the greater changes in the pharmacokinetics and biliary excretion of CPZ observed in the G-CSF- and LPS-treated group were caused by the increases in WBC number and the percentage of PMNs induced by pretreatment with G-CSF.

CPZ has been reported to be excreted primarily into the bile via the organic anion transport system (32). The lack of changes in the systemic or biliary clearance or in the biliary excretion of CPZ in the G-CSF-pretreated group indicated that pretreatment with G-CSF and neutropenia did not influence the CPZ excretory process. Moreover, treatment of G-CSF-pretreated rats with LPS led to a greater prolongation of the disappearance of CPZ from plasma than that observed in rats that did not receive G-CSF pretreatment. These results suggested that the increased percentage of PMNs induced more severe impairment of the biliary mechanism of excretion of CPZ induced by LPS administration, although the beneficial effect of G-CSF against LPS-induced hepatic toxicity has been discussed previously (8).

It is well known that the uptake of β-lactam antibiotics and BSP into the hepatocytes via the sinusoidal membrane and excretion into the bile through the bile canalicular membrane is by a carrier-mediated transport system (6). Utili et al. (35, 36) reported that the biliary excretion of BSP was decreased by *Escherichia coli* LPS in experiments with isolated perfused rat liver due to impairment of the transport process from hepatocytes to the bile. They also reported that LPS reduced the bile acid-independent bile flow rate induced by impairment of Na⁺-, K⁺-ATPase activities (37). Bolder et al. (3) have recently proposed the possibility that the decrease in the bile acid-independent flow rate is attributable to decreased activity of the ATP-dependent canalicular multiple organic anion transporter (cMOAT), since basal bile flow is predominantly driven by the secretion of anions rather than by bile acids in rats (1). Based on a consideration that CPZ is likely to be a substrate for cMOAT as well as other well-known organic anions, a relationship between the amount of biliary excretion of CPZ and the mean bile flow rate during the experimental period was examined. As shown in Fig. 5, the amount of biliary excretion of CPZ was significantly correlated with the mean bile flow rate (r = 0.891; P < 0.01). This result suggests that cMOAT activity is an important determinant of the decrease in the bile flow rate, in addition to the biliary secretion of certain organic anions including CPZ.

Roelofsen et al. (28) have reported that the LPS-induced decrease in the biliary excretion of 2,4-dinitrophenyl-S-glutathione, which is a substrate for cMOAT, was not caused by a direct effect of LPS, since the maximum reduction was observed at only 12 h after LPS injection. The present findings that the LPS-induced decrease in the biliary excretion of CPZ was enhanced in rats with neutropenia caused by G-CSF pretreatment support their findings, although a similar reduction was observed 2 h after administration of *K. pneumoniae* LPS. It is well known that LPS stimulates the immune system, macrophages, and Kupffer cells, which produce eicosanoids, and cytokines including TNF-α and interleukin-1 (14, 27). A possibility that TNF-α plays an important role in the LPS-induced decrease in sodium-dependent bile acid uptake via basolateral membranes has been discussed. In fact, based on the findings of Green et al. (9), the level of taurocholate cotransporting polypeptide mRNA, which is the sodium-dependent carrier protein for bile acid on the membranes, was reduced by direct injection of TNF-α instead of LPS administration. It has also been reported that the LPS-induced decrease in the bile flow

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bile % of dose excreted</th>
<th>CL_BILE (liters/h/kg)</th>
<th>Urine % of dose excreted</th>
<th>CL_U (liters/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70.06 ± 3.62</td>
<td>1.286 ± 0.106</td>
<td>20.10 ± 1.25</td>
<td>0.334 ± 0.047</td>
</tr>
<tr>
<td>G-CSF</td>
<td>72.97 ± 1.62</td>
<td>1.028 ± 0.038</td>
<td>21.55 ± 2.04</td>
<td>0.306 ± 0.033</td>
</tr>
<tr>
<td>LPS</td>
<td>60.02 ± 3.71&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.582 ± 0.091&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.73 ± 4.59</td>
<td>0.217 ± 0.036</td>
</tr>
<tr>
<td>G-CSF and LPS</td>
<td>43.84 ± 7.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.214 ± 0.049&lt;sup&gt;d&lt;/sup&gt;</td>
<td>36.27 ± 2.76&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.172 ± 0.033&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each value represents the mean ± standard error (n = 5 or 6).<br><sup>b</sup> Significantly different from the control group (P < 0.01).<br><sup>c</sup> Significantly different from the G-CSF-treated group (P < 0.05).<br><sup>d</sup> Significantly different from the G-CSF-treated group (P < 0.01).<br><sup>e</sup> Significantly different from the LPS-treated group (P < 0.05).<br><sup>f</sup> Significantly different from the control group (P < 0.05).
rate was prevented by passive immunization with anti-TNF-α (39). On the other hand, it has also been demonstrated that the reduction of taurocholate uptake in mixed hepatocyte membranes was not prevented by anti-TNF-α antibody pretreatment in endotoxemic animals (21). Moreover, similar suggestions have been made for the bile canalicular membrane proteins related to the transport of bile acids and organic anions, although no clear findings concerning the role of TNF-α in the LPS-induced alteration in biliary excretion of bile acids and organic anions have been obtained (9, 22, 34). Unexpectedly, the present study showed that pretreatment with G-CSF produces deleterious rather than beneficial effects on the LPS-induced decrease in the biliary excretion of CPZ, although G-CSF suppresses the TNF-α production caused by LPS treatment (8, 20, 33). A preliminary experiment also found that the plasma TNF-α levels in the LPS-treated group were significantly higher than those in the control group, whereas those in the G-CSF- and LPS-treated group were nearly equal to those in the control group (data not shown). On the basis of these observations, it is unlikely that only TNF-α plays a major role in the LPS-induced decrease in the biliary transport of organic anions including CPZ. On the other hand, Gorgen et al. (8) have demonstrated that granulocytes prepared from rats pretreated with G-CSF are primed for a phorbol ester phorbol 12-myristate 13-acetate-induced oxidative burst and for ionophore- and arachidonic acid-stimulated lipoygenase production. The results obtained in the present study therefore could suggest that factors other than TNF-α which are regulated by LPS-induced neutrophil activation are important determinants in regulating the biliary organic anion transport ability in rats with endotoxemia.

Previous experiments by Hewett et al. (15) with rats have found that the rats could be protected against LPS-induced liver injury by depleting PMNs by treating them with the Ig fraction obtained from the serum of rabbits immunized with rat PMNs (anti-PMN-Ig). The plasma AST and ALT levels 6 h after LPS administration were significantly lower in anti-PMN-Ig-pretreated rats than in control rats. Plasma total bilirubin concentrations also tended to be less in anti-PMN-Ig-pretreated rats. Moreover, they reported that fewer histopathologic lesions induced by LPS administration were found in anti-PMN-Ig-pretreated rats than in control rats. In the present study, a significant increase in the plasma AST levels was observed 2 h after LPS administration, although the increase in WBC counts did not influence the LPS-induced elevation in the AST level. In contrast, ALT levels were not changed by LPS administration. Hewett et al. (15) have shown that plasma AST and ALT activities increased between 3 and 6 h after LPS administration. The results of our preliminary test also indicated that the levels of these enzymes in plasma were highest 6 or 8 h after LPS administration. Therefore, the effect of G-CSF pretreatment against LPS-induced changes in these enzyme activities could not be assessed in this study, although the increase in WBC counts produced the deleterious effect against the biliary excretion of CPZ. On the other hand, plasma ALT activity was decreased in rats pretreated with G-CSF, regardless of LPS administration. However, the mechanism remains unknown.

In conclusion, this study shows that pretreatment with G-CSF dramatically modifies the pharmacokinetics of CPZ as a result of its deleterious effect against the K. pneumoniae LPS-induced decrease in the biliary excretion of CPZ via the organic anion transport system rather than its protective effect by suppression of TNF-α production by LPS. The results of this study may provide further information about the mechanism of the LPS-induced decrease in biliary excretion of organic anions and the role of neutrophils in LPS-induced hepatobiliary dysfunction.

ACKNOWLEDGMENTS

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